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Impact of flow-through regime on the cultivation of epithelial cells on quartz crystal resonators in micro fluidic channels

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Abstract

In this contribution the impact of culture medium flow on the cultivation of Madin-Darby canine kidney cells on quartz crystal resonators (QCRs) was studied. Cells were cultivated at different medium flow rates in a bioreactor chip containing 4 QCRs. Confluency and motility of cells were analyzed based on light microscopical pictures and impedance spectra of the resonators. Results were compared with cells cultivated in culture flasks mounted on a shaker and CFD simulation results of the bioreactor chip. Based on our studies the flow-regime in the micro fluidic channel geometry has major impact e.g. on the cell division rate.

Keywords: Quartz crystal resonators; MDCK cells; Micro fluidic channel; On-line monitoring; Electrical impedance spectroscopy

1. Motivation and objectives

Recently the online analysis of cellular signaling processes by means of quartz crystal resonators (QCRs) in flow-through regime became of interest[1]. A micro fluidic bioreactor was applied e.g. for the on-line analysis of hepatocyte growth factor (HGF) induced cell motility by means of changes in the acoustic load on a single QCR[2]. A micro fluidic biosensor array containing multiple QCRs in parallel combined with a miniaturized impedance analyzer for the parallelized online cell stimulation analysis was reported[3,4]. Madin-Darby canine kidney (MDCK-II) cells were cultivated and stimulated inside a bioreactor chip in flow-through regime, thus medium was exchanged continuously at a very low flow rate. Experimental results indicated that the flow-regime nearby the sensor surface has major impact on the development of the cell culture. The convective mass transfer is regarded as an important factor for the cellular micro environment and hence for the measured cellular response on QCRs. In this contribution the impact of cell culture medium flow in closed micro fluidic channels on the proliferation of MDCK-II cells mediated by variations in the acoustic load on QCRs is focused. Results were compared with cells cultivated in conventional flasks mounted on a shaker for inducing periodic cell culture medium flow. In addition CFD simulations based on COMSOL Multiphysics were conducted to simulate the flow velocity profile nearby the cell layer in the plane perpendicular to the sensor surface.

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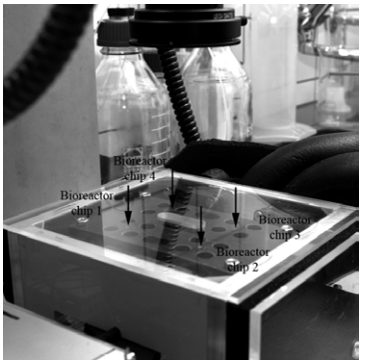
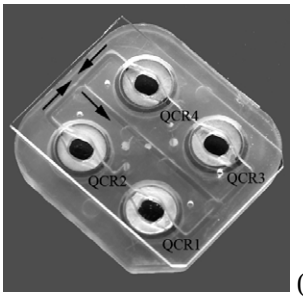
2. Micro fluidic biosensor array and methods

The micro fluidic biosensor array as published in[3] was used for our studies, see Fig. 1a. Fig. 1b shows a bioreactor chip with 4 QCRs in parallel micro fluidic channels, thus medium flow is divided into 4 identical streams.



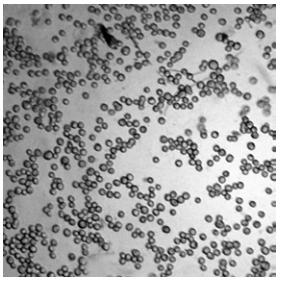
(a)



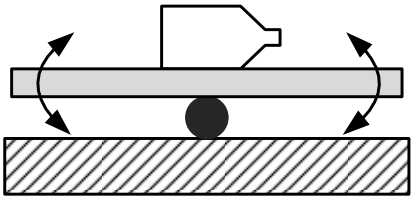
(b)

Fig. 1. (a) Micro fluidic biosensor array containing 4 bioreactor chips connected to a miniaturized impedance analyzer, a heating jacket and a light microscope for visual characterization; (b) single bioreactor chip with 4 QCRs in parallel.

The symmetry of the bioreactor chip enables an identical flow regime on each sensor surface, hence comparable cell seeding and cultivation conditions. Further details about the design and working principle of the sensor system can be found in[3]. With regard to the large number of several thousands of cells, they can be considered as randomly distributed. Dissimilar shifts in the electrical impedance spectrum can be attributed to differences in the cell distribution and hence a cell distribution dependent cellular response[5]. MDCK-II cells were seeded into a single bioreactor chip and were cultivated at different culture medium flow rates in the range of 0.5 mL/h to 2.5 mL/h over several hours in overpressure regime ~0.2 bar. Shifts in the electrical impedance spectrum of all 4 resonators were continuously measured using a miniaturized impedance analyzer. Confluency, motility and morphology of cells were analyzed based on light microscopical pictures as depicted in Fig. 2a. For comparison cells were cultivated in a conventional cell culture flask placed on a shaker located in an incubator as illustrated in Fig. 2b. The setup was used to simulate medium flow on top of the adherent cell layer in conventional cell culture comparable to the situation in the biosensor array. The major difference is that e.g. signaling proteins secreted into the extracellular medium remain in the flask, they can not be washed out. The cycle time of the shaker was set to approx. 9 s resulting in a slightly higher flow rate compared to the conditions in the chip.



(a)



(b)

Fig. 2. (a) MDCK-II cells on a QCR in the bioreactor chip at a confluency of approx. 50 %; (b) schematic of the shaker used for the analysis of cell proliferation in a conventional cell culture flask in the presence of continuous medium flow.

3. COMSOL simulations

The flow velocity profile in the bioreactor chip was simulated using the CFD module in COMSOL Multiphysics. Fig. 3 shows the simulated flow velocity in the plane parallel/perpendicular to the sensor surface.

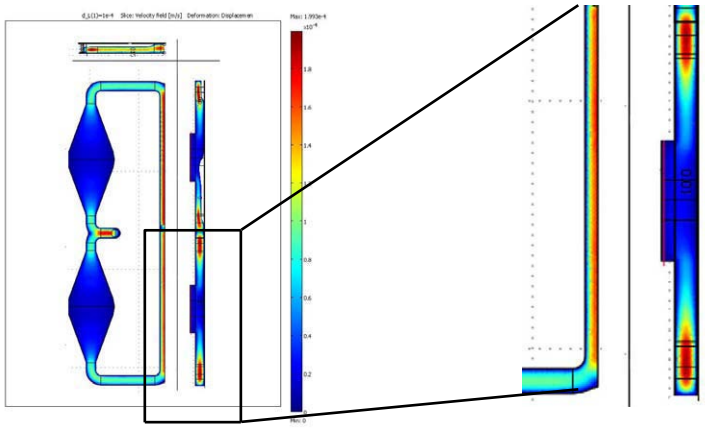


Fig. 3. Simulated flow velocity profile in the bioreactor chip based on a half model at a flow rate of 1 mL/h in parallel (left side) and perpendicular to the sensor surface (right side).

Simulation results revealed that in a reasonable range of flow rates up to several mL/h for a single bioreactor chip, the flow velocity is <<1 mm/s. Therefore it can be concluded that the corresponding shear stress on the cell layer in a range of several µm above the sensor surface can be neglected. With regard to the low flow rate the transport of species nearby the QCR due to convective mass transfer in addition to diffusion is comparably low.

4. Experimental results

When cultivating MDCK-II cells in a conventional cell culture flask on the shaker, see Fig. 2b, no significant differences in reference to a non-moving flask could be detected. Microscopical pictures showed a comparable cell morphology and cell division rate. Fig. 4 shows shifts in the series resonance frequency (f_s) and conductance maximum (G_{max}) of 4 QCRs at a comparable low flow rate of total 0.5 mL/h for a single bioreactor chip. Two hours after seeding cells into the device sensor signals represent normal adhesion of cells on the QCR, e.g. compared to[3]. In the following 10 h f_s exhibits a plateau or temporarily increases (QCR3), while G_{max} decreases slightly.

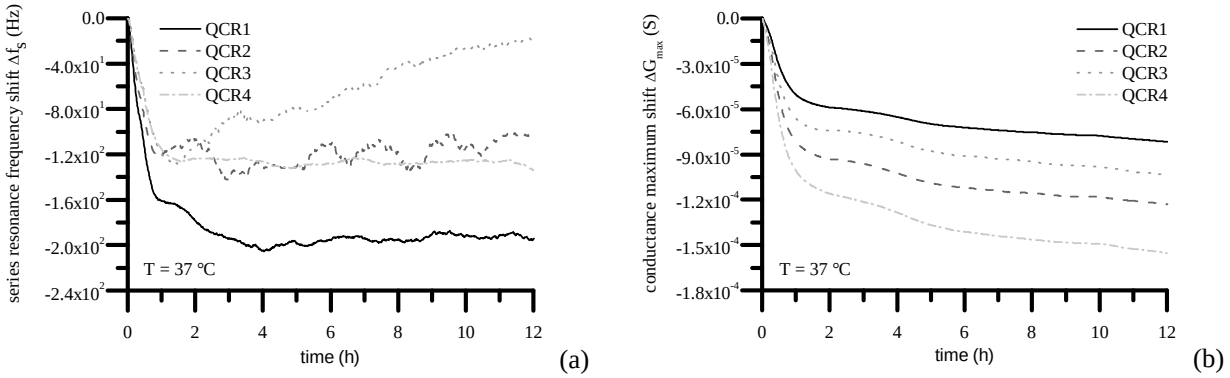


Fig. 4. QCR response of proliferating MDCK-II cells at a culture medium flow rate of 0.5 mL/h: (a) series resonance frequency shift; (b) shift in conductance maximum.

The plateau in f_s for QCR1, QCR2, QCR4 can be attributed to an almost constant number of cells adherent on the sensor surface, thus a minimum cell division rate. Results are in accordance with microscopical pictures, insofar a flow rate of 0.5 mL/h involves an insufficient culture medium supply. The micro fluidic channel of QCR3 was partly blocked by a bubble that further decreased the medium flow and thus increased the number of dead cells nearby the bubble, but still sticking to the surface. Fig. 5 shows the cellular response at a comparable high flow rate of 2.5 mL/h. In the first two hours sensor signal shifts are comparable to the curves shown in Fig. 4.

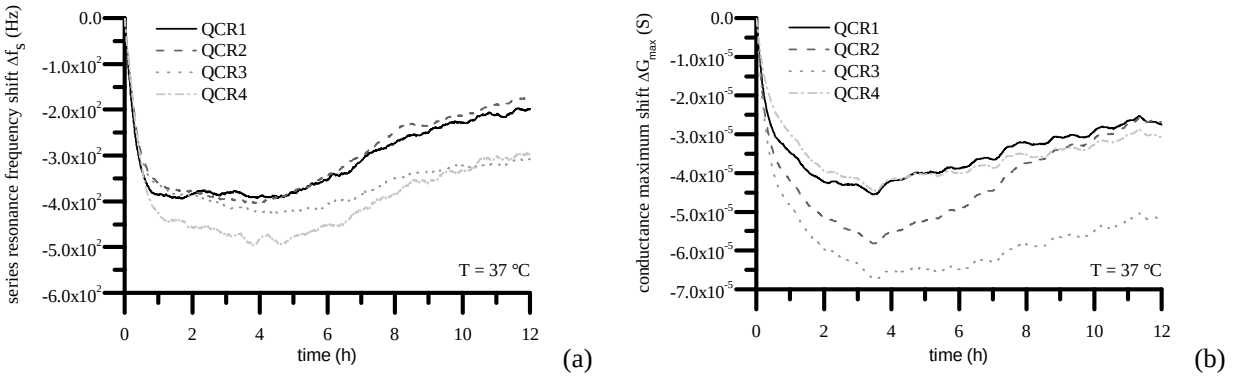


Fig. 5. QCR response of proliferating MDCK-II cells at a culture medium flow rate of 2.5 mL/h: (a) series resonance frequency shift; (b) shift in conductance maximum.

In contrast after 3 to 5 h cells start to die and detach from the surface. Short term alterations of signals in both experiments can be mainly attributed to cell motility, while deviations in between QCRs are mainly due to a slightly different confluency and cell distribution. When comparing sensor signals with cell cultivation in flasks on a shaker, it can be concluded that fluid movement is not the governing factor for the cellular response at flow rates >>1 mL/h.

5. Summary and conclusion

In this contribution the impact of culture medium flow on the cultivation of MDCK-II cells on QCRs in closed micro fluidic channels in reference to a conventional cell culture flask was studied. COMSOL simulations depicted that shear stress due to fluid flow does not play a role in the bioreactor chip. When decreasing the flow rate in reference to 1 mL/h, first the cell division rate is reduced, but cells are still alive. The cellular response by means of alterations in the acoustic load is dominated by an insufficient nutrient supply and removal of metabolic waste products. When increasing the flow rate up to several mL/h, the convective mass transfer interferes with the cellular micro environment followed by cell death. With regard to the results from cell cultivation in flasks placed on a shaker, we suppose that above a certain flow rate a washing out of species secreted in the extracellular medium due to convective mass transfer leads to dead cells. Based on our results the flow-regime and thus the flow rate has major impact e.g. on the cell division rate of MDCK-II cells. It can be stated that for the proliferation of MDCK-II cells in flow-through regime only a limited range of flow rates can be used for a certain micro channel geometry.

Acknowledgements

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